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Title: Apparatus for the noninvasive determination of the oxygen turnover and other parameters in biological tissues

Description:

The invention relates to the determination of the local oxygen turnover, the oxygen consumption, the oxygen content and the locally transported amount of oxygen in perfused tissues using an optical sensor as set forth in the preamble to claim 1.

Oxygen is of elemental importance for almost all cells in biological tissues. In perfused tissues, oxygen is mostly transported in a form bound to hemoglobin, which is incorporated in the erythrocytes, from the lung to the oxygen-consuming cells. Arterial hemoglobin in the systemic circulation which has undergone saturation in the lung is almost 100% saturated with oxygen. In the peripheral tissue, the oxygen is then delivered along the capillaries to the cells. Correspondingly lower saturations of hemoglobin with oxygen are measured in the tissue regions at the venous end of the capillary and in the downstream venules and veins.

Noninvasive measurement of the oxygen content in tissue according to the prior art is possible only with very elaborate methods such as NMR (nuclear magnetic resonance). In addition, dynamic measurements of the oxygen consumption or monitoring of the oxygen removal through tissue respiration are not as yet possible with NMR methods.

The energy metabolism of the cells, which is closely linked to the oxygen uptake, can be determined only invasively, by taking samples of tissue or body fluids followed by biochemical laboratory analysis of the metabolic products. Thus, no dynamic measurements of the oxygen consumption or

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The arterial saturation of hemoglobin  $\text{SO}_2$  (%) in the arterial system can be determined by so-called pulse oximeters, but these do not customarily allow the capillary-venous hemoglobin saturation levels, the hemoglobin concentration in the measured volume or the blood flow to be determined. The methods used in pulse oximetry are based on findings from cuvette photometry and use only a few individual wavelengths, so that a change in the measured volume and the recording of changes in scattering or absorption cannot be taken into account. This means that these measurement methods are associated with considerable measurement uncertainties. The advantage of these instruments to date is that they are very convenient and easy to handle.

Two methods offer a possible approach to quantifying the light path and thus the effective measured volume:



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The propagation of light in biological media, scattering suspensions, tissue sections and intact tissues and cell structures is determined by their optical properties. The propagation of light is moreover determined by the two basic phenomena of light absorption and light scattering. The absorption of light or the attenuation in the sense of converting the light energy into light of a higher wavelength

In contrast to this, light loses no energy through elastic scattering. The incident electromagnetic wave which interacts with the scattering center radiates the energy again, after excitation by the incident light wave, into the various spatial angles. During this, the light retains its wavelength, and only the direction of propagation of the light is altered by the scattering centers. At the molecular level, the physical process is to be conceived as excitation of the scattering particles by the incident light wave and thereafter emission of the energy back into space at the same wavelength. The directions in which the light energy is radiated then depend on the geometry, the shape and the electromagnetic distribution of the electron shells of the molecule, and on the structure.

The incident, directed beam is converted on the path through the tissue into diffuse radiation and adds up to forward scattering of the excited scattering centers. After scattering, which is in some circumstances multiple, a small part of the incident light intensity returns to the surface. The radiation backwards from all the scattering centers adds up to the backscattering. The light guides on the organ surface can detect only light which has been backscattered and not quenched by absorption on its light path. However, the only light detected by the light guide on the organ surface is that backscattered within the light guide aperture. This

conception of the propagation of light is formally described in the radiation transport theory.

It is an object of the invention to determine by measurement techniques the hemoglobin oxygenation and the hemoglobin concentration, and measurements derived therefrom, and to propose an instrument for measuring the oxygen content and other related parameters (see Table 1).

This object is achieved according to the invention by an apparatus having the features of claim 1. Dependent claims relate to embodiments of the invention. A suitable name for the instrument according to the invention would be AbTisSpec, an acronym for absorption tissue spectrophotometer.

Thus, the invention proposes an apparatus which determines the oxygen content and data derived therefrom using an optical sensor to be placed on the tissue. It comprises one or more light sources which send light through optical fibers to the sensor, one or more detectors which receive light backscattered by the tissue through optical fibers, and an evaluation unit which obtains information about the oxygen content from the backscattered light. An apparatus of this type is described for other purposes for example in EP 771 546 A2, the whole of the disclosure and technical design of instruments of which is here made subject-matter of the invention.

The light source preferably used is a white light source and/or a laser source. Whereas the white light source introduces the light into the tissue whose backscattered portions later undergo spectral analysis, the laser light source introduces a monochromatic light whose backscattered portion has a measurable frequency shift so that a Doppler measurement and thus a rate measurement are possible.

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A white light source (or various broad-band LEDs) is used for the illumination. A light source with a high illuminated field density and a spectrum of maximum whiteness and smoothness is crucial. The light backscattered from the tissue is spectrally analyzed by a polychromator, amplified and subsequently used as wavelength-dependent light intensity pattern for the evaluation.

The wavelength range from 500 to 650 nm (VIS) is particularly suitable for measurements close to the surface. By contrast, the wavelength range from 600 nm to 900 nm (NIR) is particularly suitable for depth-selective measurements even at relatively large depths because the effective depth of penetration of the light in the NIR wavelength range is greater than in the aforementioned wavelength range. In addition, different measured volumes are specified by the sensor geometry through different spacings of illumination and detection regions. The combination of the selection of detection spacings and appropriate wavelength ranges can thus be clearly defined and permits clear demarcation of individual measured volumes from one another. For calculation of the saturation values there is evaluation of the spectral shape of the spectra, for example by a shape recognition and mixing method or of an approximation of the diffusion equation. The measurements obtained according to the invention are, depth-selectively, the saturation of the hemoglobin with  $O_2$  in the capillary venous tissue bed.

The measured volume is determined by a novel method, namely by measuring an intensity gradient on the surface in combination with determination of the absorption and scattering coefficients from the Doppler measurements, and of the spectrometric measurements.

The amount of hemoglobin can be obtained from the evaluation of the spectrometric data. The absorption caused by the

A large number of measured quantities is required to describe the complete oxygen supply situation (see Table 1). The arterial saturation and, independently thereof, the capillary venous saturation of the hemoglobin must be determined. It is possible from the difference between the arterial saturation and the saturation of the hemoglobin in the capillaries, venules and veins then to determine the  $O_2$  turnover or the oxygen uptake. Combination of this differential amount with the blood flow values affords the  $O_2$  consumption in the investigated tissue volume, which can thus be determined locally.

1. Depth-selectively the saturation of hemoglobin  $\text{SO}_2$  [%] in the arterial system.
2. Depth-selectively the saturation of hemoglobin  $\text{SO}_2$  [%] in the capillary venous system.
3. Depth-selectively the amount of hemoglobin in the measured volume, from the absorption coefficients.
4. Size of the measured volume, resulting from the surface gradient measurement, the scattering coefficients and the anisotropy factors, or from models of the diffusion theory.
5. Depth-selectively the amount of blood flowing and the blood flow rate.
6. The local tissue temperature.

The combination of these measured quantities and the detection by an integrating sensor which guarantees measurements in the same area permits measurement of the local oxygen turnover of perfused tissue.

It is possible to provide as evaluation unit a spectrometer, a spectroscopy, a laser Doppler spectroscopy, a tissue spectrometer, a tissue spectroscopy, a pulse oximeter and/or a temperature gauge, each alone or in any desired combination.

It is thus possible to determine

- a) the local oxygen content,
- b) the local oxygen consumption in arterial/venous mixed tissue,
- c) the oxygen consumption rate in arterial/venous mixed tissue,
- d) the total amount of blood,
- e) the local oxygen transport capacity,
- f) the locally transported amount of oxygen,
- g) the oxygen turnover in arterial/venous mixed tissue,
- h) the oxygen turnover rate in arterial/venous mixed tissue and/or
- i) the local tissue oxygen partial pressure in perfused tissues.

In one embodiment of the invention, information is obtained from different depths by the selection of the wavelength range and of the detector-transmitter separation. Thus, measurements can be obtained both from the tissue surface and from deeper regions.

The apparatus according to the invention is suitable not only for detection of hemoglobin and of the measurements which can be derived therefrom, but also for measuring the content of tissue pigments such as cytochromes, myoglobin, melanin, bilirubin or other pigments present in the tissue, and data derived therefrom. The same optical sensor can be used for placing on the tissue for this purpose. Likewise the one or more light sources which send the light through optical fibers to the sensor. In addition, one or more detectors which receive light backscattered from the tissue through optical

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The light source which can be provided in this apparatus is also a white light source and/or a laser source.

Information from different depths can be obtained by the selection of the wavelength range and of the detector-transmitter separation.

On use of an additionally depth-selective sensor or of a depth-selective evaluation it is possible to generate a three-dimensional image of the recorded measurements. This permits a "view" into the interior of an organ or of a tissue and permits layer-wise representation of the relevant data.

Crucial for these optical measurements according to the invention is direct contact of the sensor with the organ surface. Only this makes it possible to carry out measurements



- Fig. 14 the extinction as a function of the hemoglobin concentration in tissue perfused in isolation,
- Fig. 15 backscattering functions in the x and z directions
- Fig. 16 a scattering cuvette,
- Fig. 17 detection depths,
- Fig. 18 transfer function of the x to z gradients,
- Fig. 19 the model of a measured volume,
- Fig. 20 two absorption spectra,
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- Fig. 22 a two-dimensional measurement distribution,
- Fig. 23 an apparatus for recording three-dimensional measurement distributions and
- Fig. 24 cytochrome spectra, oxidized cytochromes and reduced cytochromes measured in a suspension of mitochondria.

Fig. 1 shows diagrammatically, viewed from below, that is to say starting from the tissue, one embodiment, merely by way of example, of an integrated sensor S or measuring head as can be used in the apparatus according to the invention. In this case it contains for the illumination the white light source W, the laser light source L and a plurality of detectors, where DD designates the detectors for Doppler signals and DR designates the detectors for backscattered intensities. The linear arrangement shown is merely by way of example. Many other arrangements are possible, with the detectors DR, which are more remote from the light source W, receiving light which has penetrated into deeper regions of the tissue and has been backscattered there, as described in EP 771 546 A2. A temperature sensor DT is additionally shown.

According to the invention, photons from a coherent monochromatic light source L and, preferably, additionally photons from one or more broad-band white light sources W are beamed through the integrated sensor system S into the tissue in a first region. The re-emerging photons are detected (DD,

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DR) at various distances from this first region. In spatial alternation there is detection of the light for the laser Doppler evaluation and for the spectroscopic or spectrometric evaluation. The design of the integrated sensor is depicted by way of example in Fig. 1.

The depth-selective laser Doppler measurements can be carried out with an apparatus as described in EP 771 546 A2.

According to the invention, Fig. 2 shows a specific form of the applicator used in particular for endoscopic measurements in the gastrointestinal region. The advantage of this fiber arrangement is the compact construction and easily reproducible geometry. As above, W represents the point of illumination with a white light source, and L represents the illumination with the laser source. In spatial alternation, the light is detected for the laser Doppler evaluation DD and for the spectroscopic or spectrometric evaluation DR. This sensor allows measurements in two detection depths in each case. The temperature sensor DT can optionally be arranged in the center of the sensor, or another fiber can.

Fig. 3 shows an arrangement from Fig. 2 which has been slightly modified according to the invention for applications with two separations for the laser Doppler measurements DD and evaluation DR of the spectrometric and/or spectroscopic measurements in one separation, but with larger total cross sections, because of the inclusion of the intensities of three detection fibers. Fig. 2 shows a white light source W and a laser light source L.

The fiber arrangement in Fig. 4 shows an apparatus according to the invention for determining the primary signals, obtained through a tissue spectrometer, of the arterial oxygen saturation, of the local oxygen saturation of the hemoglobin,

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A white light source W (or various broad-band LEDs) for example are used for the illumination. A light source with high illuminated field density and a spectrum of maximum whiteness and smoothness is crucial. The light backscattered from the tissue is - spectrally analyzed by a polychromator - amplified and subsequently used as wavelength-dependent light intensity pattern for the evaluation.

According to the invention, two wavelength ranges are defined and distinguished.

The wavelength range from 500 to 650 nm (VIS) is particularly suitable for measurements near the surface, and the wavelength range from 580 to 900 nm (NIR) is particularly suitable for depth-selective measurements also at greater depths and in larger volumes, because the effective depth of penetration of the light in the NIR wavelength range is greater than in the aforementioned wavelength range. A detector unit which has particularly high sensitivity in the wavelength range near the infrared is necessary for detecting hemoglobin levels in the

Selective measurements near the surface are possible according to the invention firstly owing to the combination of the selection of the wavelength range from 500 to 650 nm and of the light guide separation of less than 2 mm. Secondly, measurements in the macrovolume and in great depths of detection are possible owing to the selection of the wavelength range from 650 to 900 nm together with light guide separations of more than 2 mm.

The detector unit with polychromator is the core of the tissue spectrometer according to the invention. A highest possible quantum yield and, resulting therefrom, a high detection frequency are desired. The greatest demands on the speed of detection are made on application of the method in cardiology, because the fastest physiological reactions are to be expected here. The saturation of hemoglobin  $\text{SO}_2$  pulsates on the myocardium with the heart rate. The critical  $\text{SO}_2$  values are to be expected at the end of systole because myocardial perfusion is greatly restricted during the contraction because of the high pressure in the ventricle. The heart rate is about 1/sec, with a systolic contraction duration in the region of 100 ms. This results in a maximally necessary scan frequency of 10 msec in order to be able to resolve this interval sufficiently. All the other physiological processes in the

human body take place correspondingly slower and can be detected with lower scan frequencies. Another advantage of high scan rates is the increasing security of operation, which ensure "blur-free" recordings.

The following table summarizes the oxygen parameters for describing the local supply situation which are possible with the integrated sensor concept according to the invention. In the table, a cross has been put at the place at which a signal is required either from the tissue spectrometer values, the laser Doppler data, the pulse oximeter values or from the temperature values in order to be able to determine therefrom the corresponding oxygen parameter. The definition of the local oxygen supply quantities mentioned are explained in more detail hereinafter.

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Oxygen parameter	Tissue spectrometer				Laser Doppler			Pulsoxi-meter	Temp.
	SO <sub>2</sub> cap.-ven.	Hb <sub>conc</sub>	Measured vol. or intensity gradient	Hb <sub>conc</sub> /Vol	Blood flow	Velocity v	Pulsa-tility	SO <sub>2</sub> arterial	T
Oxygen content	X		x	x					
Oxygen consumption	X		x	x			x	x	
Total amount of blood			x	x					
O <sub>2</sub> transport capacity		x			x				
Amount of O <sub>2</sub> -transported	x	x			x				
Oxygen consumption rate	x	x					x	x	
Oxygen turnover	x		x	x		x	x	x	
Oxygen turnover rate	x	x			x		x	x	
Tissue pO <sub>2</sub>	x								x

Table 1:      Compilation of the methods by which it is possible with use of the tissue spectrometer, the laser Doppler spectroscope, the pulse oximeter and/or a temperature gage to determine the clinically relevant blood and oxygen supply quantities on integration or partial integration of the sensors in a combined measuring head and integrative data analysis.

The various oxygen parameters are derived, and the formal relationships of the methods according to the invention are defined, hereinafter.

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Measurement of the oxygen content in the blood requires the amount of hemoglobin in the investigated tissue volume to be determined and the saturation of the hemoglobin present to be determined. It is therefore indispensable also to be able to determine quantitatively the illuminated tissue volume, because the amount of hemoglobin must be related to the value for the volume. The size of the illuminated or measured volume is crucially determined by the sensor geometry and the basic physical-optical parameters of the tissue, which are formulated in the form of absorption coefficients  $\mu_a(\lambda)$  and scattering coefficients  $\mu_s(\lambda)$  in the spectral range used of the light sources W and L. The relationships mentioned are derived in formal relations in the form of mathematical formulae stepwise below:

The  $O_2$  content in the blood can thus be determined formally by the following formula:

$$O_2 \text{ Content} = Hb_{\text{Amount}} \cdot SO_2 \cdot H$$

Thus, the measurement problem is divided into two different tasks. On the one hand, determination of the amount of hemoglobin  $Hb_{\text{amount}}$  and, on the other hand, determination of the saturation of hemoglobin  $SO_2$  with oxygen. Hüfner's number  $H$  produces the relationship between hemoglobin content and maximum oxygen content. Calculation of the amount of hemoglobin is described in turn in a following paragraph.

The oxygen turnover or the oxygen consumption in the tissue can be formally described by:

$$O_2 \text{ Consumption} = O_2 \text{ Content arterial} - O_2 \text{ Content venous}$$

On the assumption that the amount of blood arterially and venously remains the same on the basis of the continuity equation, the formula can be simplified to:

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$$O_2 \text{ Consumption} = H \cdot (Hb_{\text{Saturationarterial}} - Hb_{\text{Saturationvenous}}) \cdot Hb_{\text{Amount(in measured volume)}}$$

It is known from the literature that the arterial blood volume in the tissue is typically less than 5%. It is thus sufficient to determine only the amount of hemoglobin in the capillary venous system without causing large errors.

If a restriction is made, in place of determination of the amount of  $O_2$  which has been consumed, to the oxygen consumption rate, an indicator of the ratio of arterial to venous consumption, the following equation results therefrom for the determination:

$$O_2 \text{ Consumption rate} = (SO_2 \text{ arteriall} - SO_2 \text{ venous}) \cdot Hb_{\text{conc.}}$$

The  $Hb_{\text{conc.}}$  can be determined in the same manner as defined below. It is evident from the explanations hereinafter that  $Hb_{\text{conc.}}$  is proportional to the extinction (or else the optical density) based on the particular measured volume.

It is not always possible in the tissue to combine the measured arterial saturation with the relevant capillary venous hemoglobin saturation. Strictly speaking, this is the case only when the continuity equation is satisfied in the appropriate tissue volume. It is thus important for arterial and capillary venous systems to be present in the measured volume under consideration and for the arterial pulse to be detectable.

However, it is possible to determine locally for every tissue volume a quantity which is to be referred to as the transported amount of oxygen or local oxygen transport capacity. These quantities can be measured with the novel integrated sensor concept.

The local oxygen transport capacity is determined by the number of moving erythrocytes locally present and their

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Since laser Doppler instruments calculate a value which corresponds to a relative perfusion *Blood Flow*, it is also possible in an approximate solution for relative values to employ the following equation. The blood flow in this case indicates a measure of the number of moving erythrocytes multiplied by their speed and thus represents a measure of a volumetric flow. The result is thus:

The locally transported amount of oxygen is determined by the number of erythrocytes locally present multiplied by the flow velocity of the erythrocytes. This product is calculated by the laser Doppler instruments as a value which is referred to as blood flow. Blood flow multiplied by Hüfner's number and the local oxygen saturation of hemoglobin results in the transported amount of oxygen in the area investigated.

The oxygen turnover is proportional to the amount of  $O_2$  which is consumed in a defined area of tissue. The consumed amount of  $O_2$  results from the difference between the amount of oxygen transported into the tissue (arterially) from the amount transported out again on the venous side. The quantitative oxygen turnover can be calculated as shown from:

$$O_{2\text{turnover}} = (SO_{2\text{arterial}} - SO_{2\text{venous}}) \cdot \frac{H \cdot Hb_{\text{Amount}} \cdot v_{\text{Blood}} \cdot \text{Amount}_{\text{Erys, moving}}}{\text{in the measured volume}}$$

The same assumptions, approximations and abbreviations already assumed above apply in this case.

Since laser Doppler instruments calculate a value which corresponds to a relative perfusion *Blood Flow*, it is also possible in an approximate solution for relative values to employ the following equation.

$$O_{2\text{turnover rate}} = (SO_{2\text{arterial}} - SO_{2\text{venous}}) \cdot H \cdot \text{Blood Flow}$$

A description of the calculation of the primary information follows:

For calculation of the hemoglobin saturations  $SO_2$  it is possible, for example, to analyze the spectral shape of the spectra by a shape recognition and mixing method. The measurements obtained are the saturation of hemoglobin with  $O_2$  in the capillary venous tissue bed (described in W. Dümmler, Thesis, University of Erlangen, 1998).

In the present specific task of determining Hb spectra by tissue measurements, the absorption  $A$  is represented as total formed from the fundamental absorption  $A_o$  and the combined absorption portions of 0% and 100% oxygenated hemoglobin. The spectral absorption coefficients are expressed by the specific extinctions of oxygenated Hb,  $\epsilon_{ox}^{Hb}$  and deoxygenated extinction coefficients of Hb,  $\epsilon_{deox}^{Hb}$  in the following equation:

$$A(\lambda) = A_o + C_{ox} \cdot \epsilon_{ox}^{Hb}(\lambda) + C_{deox} \cdot \epsilon_{deox}^{Hb}(\lambda)$$

The coefficients  $C_{ox}$  and  $C_{deox}$  indicate the combination portion from which each measured spectrum can be composed according to its degree of oxygenation.

The scattering  $S$  is approximated in a first approach as a first order wavelength-dependent function consisting of the linear combination of fundamental scattering  $S_0$  and wavelength-dependent scattering portion  $S_1$ .

$$S(\lambda) = S_0 + \lambda \cdot S_1$$

The method described above is used to bring the measured spectra to the form  $A/S$  and equate them to the model approach, see right-hand side of the following equation.

$$\frac{A}{S} = \frac{A + C_{ox} \cdot \epsilon_{ox}^{Hb}(\lambda) + C_{deox} \cdot \epsilon_{deox}^{Hb}(\lambda)}{S_0 + \lambda \cdot S_1}$$

The hemoglobin saturation is determined by iterative determination of the coefficients  $\frac{A_0}{S_0}, \frac{C_{ox}}{S_0}, \frac{C_{deox}}{S_0}, \frac{S_1}{S_0}$  by Newton's and the least squares methods and subsequent quotient formation:

$$SO_2 = \frac{C_{ox}}{C_{ox} + C_{deox}}$$

The hemoglobin saturation can thus lie only in the range of values from 0% to 100%. The accuracy of calculation depends on the quality of the tissue model. The tissue model presented above can be extended at any time, and it is thus possible to replace the basic absorption  $A_0$  by tissue-specific basic spectra  $A_{Tissue}(\lambda)$  which can be taken from an organ table.

At the start of each measurement, in order to improve the spectrometer values a dark spectrum should be recorded in

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The tissue model presented by W. Dümmler (Thesis, University of Erlangen, 1998) can be extended according to the invention so that the specific organ spectra  $A_{tissue}(\lambda)$  are included directly in the model.

$$\frac{A}{S}(\lambda) = \frac{A_{tissue}(\lambda) + C_{ox} \cdot \epsilon_{ox}^{Hb}(\lambda) + C_{deox} \cdot \epsilon_{deox}^{Hb}(\lambda)}{S_0 + \lambda \cdot S_1}$$

The specific tissue spectra can be obtained for each organ as a typical average spectrum during hemoglobin-free perfusion.

It has been realized from many measurements that backscattered hemoglobin spectra are distorted by erythrocytes and, in particular, compressed compared with spectra recorded in the transmission configuration of light source and detector. The properties of the individual spectrometer components play no part in this. The amplitudes of the hemoglobin spectra measured in the reflectance configuration should be similar to the amplitudes of the Hb/HbO<sub>2</sub> reference spectra. This again means that suitable and comparable Hb/HbO<sub>2</sub> reference spectra are required in each case. The main reason for the compression of the spectra lies, according to the current state of knowledge, in the differences of the measured volumes of the various hemoglobin absorptions. Light with wavelengths between 540 nm and 580 nm experiences greater attenuation than in the adjoining wavelength ranges and therefore penetrates less deeply into the tissue. Light of the more weakly absorbing wavelengths of the hemoglobin spectrum by contrast penetrate more deeply into the tissue and therefore experience, in absolute terms, a greater degree of attenuation than would be concluded directly from the extinction. It was possible to conclude this relationship directly from the measurements of the depths of penetration in the bilayer models (see A. Krug, Thesis, Erlangen University, 1998).

A bilayer model which is composed of a scattering suspension (for example Intralipid®, cells or tissue layers) disposed on

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top of a total absorber, such as, for example, marking ink, separated by a PVC film only a few  $\mu\text{m}$  thick, is described in Fig. 8. Two light guides extend into the suspension; the first guides light in and the second picks up backscattered light. The arrangement is used for determining the 90% depth of penetration, which will also be referred to as the detection depth.

Detection depths in $\mu\text{m}$ for marking ink as background absorber			
Wavelength	542nm	628nm	760nm
IL 2%	920	1000	1120
IL2%+0.25 g Hb/dl	320	580	880

  

Detection depths in $\mu\text{m}$ for black PVC as background absorber			
Wavelength	542nm	628nm	760nm
IL 2%	1000	1040	1060
IL2%+0.25 g Hb/dl	380	660	920

Table 2: Calculation of the detection depths, recorded with 200  $\mu\text{m}$  quartz fibers in Intralipid suspension.

the measured spectra are rectified according to their hemoglobin concentration by relating the extinctions to the effective measured volume in each case, or a whole series of HbO<sub>2</sub> reference spectra are generated with different hemoglobin concentrations and different degrees of distortion and are made available to the evaluation algorithm. The former solution is to be preferred because, in this case, standard hemoglobin spectra from cuvette measurements can be used, and the determination of the measured volume can additionally be used for quantitative calculation of the hemoglobin concentration.

The amount of hemoglobin is determined as described in the literature by the following equation:

$$Hb_{Amount} = C_{Hb} \cdot V_{Mea}$$

It is clear from the above formula that to determine the amount of hemoglobin it is necessary for the two quantities of the hemoglobin concentration  $C_{Hb}$  (especially in the VIS) and the measured volume  $V_{MEAS}$  to be determined by measurement techniques and calculated.

Various theoretical approaches can be used to determine the amount of hemoglobin in the particular measured volume. In the simplest case, the hemoglobin concentration is determined using the Lambert-Beer law (see next formula):

$$Ext. = \log \frac{I_0}{I} = C_{Hb} \cdot \epsilon_{Hb}(\lambda) \cdot d$$

The extinction  $Ext.$  is calculated from the logarithm of the light intensity  $I_0$  beamed into the object, related to the light intensity  $I$  emerging from the object. According to Lambert-Beer, the extinction depends on the hemoglobin concentration  $C_{Hb}$ , the wavelength-dependent absorption coefficient  $\epsilon_{Hb}$  and the cuvette path length  $d$ .

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The  $Hb_{conc.}$  is to be determined in the same manner as described above.  $Hb_{conc.}$  is proportional to the extinction (or else the optical density) relative to the particular measured volume.

$$Hb_{conc.} = K_i \cdot \frac{OD.}{V_{Meas.}} = K_i \cdot \frac{\log \frac{I_o}{I}}{\epsilon_{Hb} \cdot V_{Meas.}}$$

Determination of the measured volume is described hereinafter.

Another possibility for determining the hemoglobin concentration is provided by the radiation transport equation. In its general form, however, no complete solution is possible and, for this reason, it cannot be manipulated particularly well. Therefore the diffusion approximation of the radiation transport equation is frequently used in tissue spectrometry.

The equations derived on the basis of the diffusion approximation are introduced below. Using the diffusion approximation, an x gradient in the reflectance configuration of the light guides is formally described as:

$$I(x) = \frac{3P_o}{16\pi^2} \frac{\exp\left(-x\sqrt{3\mu_a(\mu_a + \mu_s')}\right)}{x} (\mu_a + \mu_s')$$

From this it is possible by condensing to two coefficients  $C_1$  and  $C_2$

$$\begin{aligned} C_1 &= \frac{3P_o}{16\pi^2} (\mu_a + \mu_s') \\ &= \frac{3P_o}{16\pi^2} (\rho\sigma_a + \rho\sigma_s(1-g)) \end{aligned}$$

and

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$$\begin{aligned}
C_2 &= \sqrt{3\mu_a(\mu_a + \mu_s')} \\
&= \sqrt{3\rho\sigma_a(\rho\sigma_a + \rho\sigma_s(1-g))} \\
&= \rho \cdot \sqrt{3\sigma_a(\sigma_a + \sigma_s(1-g))}
\end{aligned}$$

to condense the description of the gradients to:

$$I(x) = C_1 \frac{\exp(-C_2 x)}{x}$$

From the coefficients  $C_1$  and  $C_2$  found it is possible subsequently to determine  $\mu_a$  and  $\mu_s$ . The coefficient  $\mu_a(\lambda)$  represents the absorption coefficient of the tissue, from which it is possible, with appropriate approximations or after neglecting other absorbers in the tissue, to determine the hemoglobin concentration in the tissue.

Quantitative determination of the local hemoglobin concentration in the microvolume by evaluating the backscattered spectra is a complex task. Various research groups have investigated the optical properties of various tissues. It has emerged that the scattering coefficient  $\mu_s$  is at least 10 times larger than the absorption coefficient  $\mu_a$ . Accordingly, the backscattered amount of light is primarily determined by the scattering properties of the tissue investigated.

Fig. 9 shows three spectra, namely the spectrum of the instrument function  $I_{\text{inst.}}(\lambda_i)$ , the spectrum of an ideal scatterer  $I'_o(\lambda_i)$  and a measured hemoglobin spectrum  $I_m(\lambda_i)$ .

The two statements which follow are important for developing the subsequent determination method for calculating the hemoglobin concentration:

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1. The only light intensities which can be measured on the tissue surface are those reflected back by scattering in the tissue.
2. If there is an absorber such as hemoglobin in the tissue it attenuates the light on the path into the tissue between the scattering events and on the path back to the detector light guide.

It is possible to conclude from the two statements that, at a wavelength at which the absorption is negligibly small, the measured light intensity is determined only by the backscattering. At all other wavelengths where the absorption is not negligible, the light is attenuated by the absorber, and the intensity is therefore less than the undisturbed backscattered intensity.

Thus, a novel evaluation method for determining the Hb amplitudes of tissue spectra has been developed according to the invention. The first part has already been published in the thesis by A. Krug (1998, Erlangen University). The method in which the intermediate results of the Hb determination are related, by extracting hemoglobin amplitudes from the backscattered spectra, to the value for the measured volume, described by a second method, at the same measurement point is novel. This results according to the invention in hemoglobin concentrations which are continually related to the current measured volume. The intermediate value of the relative hemoglobin concentration can also be found from solutions of the diffusion equation.

Fig. 9 depicts uncorrected spectra. The curve  $I_{\text{inst.}}(\lambda_i)$  shows the instrument function or the optical error function of the tissue spectrometer. All measured spectra must be corrected by comparison with this spectrum in order to eliminate the instrument-specific falsifications of the measured spectra.

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If the pure backscattered intensity  $I'_o(\lambda_i)$  is known, the proportion of light attenuation by the absorber can be found from the difference between the pure backscattered intensity and the measured intensity  $I_m(\lambda_i)$ . The formal relationship can be expressed by

The unattenuated backscattered intensity  $I'_0$  is obtained in backscattering photometry if the absorber concentration in the tissue equals zero. That is to say if only scatterers are present in the tissue.

Fig. 10 illustrates that the difference between  $I'_o(\lambda_i)$  and  $I_m(\lambda_i)$  corresponds to the absorption of light at this wavelength. Thus, the quotient

$$\log [I'_{\alpha}(\lambda_i) / I_m(\lambda_i)]$$

To calculate the hemoglobin concentration, the area of the extracted hemoglobin amplitudes is integrated, and an absorption is calculated therefrom for each spectrum. The integrated for values of oxygenated and deoxygenated spectra differ, however. For this reason, in the second stage of development, an oxygenation-dependent correction of the extracted hemoglobin amplitudes or of the area absorptions was introduced. The basic idea of this correction is to establish the areas of the differently oxygenated spectra and to carry out a correction for the different levels of oxygenation. It was found that the area of a fully oxygenated Hb spectrum, for example in the wavelength range 500 - 630 nm is 16% larger than the area of a fully deoxygenated spectrum (see Fig. 10). The spectra in the literature, of Assendelft, 1970, were used to establish these corrections.

Fig. 11 shows an application of the optical oxygen sensor according to the invention together with a temperature sensor as ear sensor. Fig. 11 shows the auditory canal into which this special sensor head has been introduced, with eardrum. It can, for hygienic reasons, be provided with a transparent protective film. This hygienic cap and the sensor have a special shape adapted to the auditory canal and a type of mechanical stop which prevents perforation of the eardrum. The length of the sensor up to the mechanical stop is about 25 mm.

The temperature measurement methods which are suitable and preferred here are non-contact methods such as infrared temperature measurement, or the methods for temperature measurement in the space of a closed auditory canal or the inner ear by NTC or similar temperature measurement methods.

For optical measurements of the perfusion parameters also in a microvolume it is important to develop appropriate applicators which, on the one hand, guarantee that the sensor lies on and is thus in direct contact with the tissue and, on the other hand, permit a determination when the contact pressure on the tissue is too high. The contact pressure must not exceed a certain level because, otherwise, the perfusion of the capillaries near the surface is impaired by the pressure of the sensor and thus may lead in some circumstances to an incorrect measurement.

Suitable temperature sensors are non-contact methods such as infrared temperature measurement, or methods for temperature



measurement in the space of the closed outer ear or NTC or similar temperature measurement methods.

In the embodiment of the applicator shown in Fig. 11, only reflection measurements are possible because the applicator is not in contact with the eardrum. Single-channel measurements with the tissue spectrometer, with the pulsatile tissue spectrometer, with the pulse oximeter, with the laser Doppler and/or the temperature probe are sufficient for reflection measurements.

For imaging tissue parameters on the eardrum, in accordance with Fig. 21 or Fig. 23, the ear stopper must be extended and must be placed directly on the eardrum. For 2D and, in particular, 3D imaging it is again necessary to carry out backscattering measurements in place of reflection measurements.

Fig. 12 shows an embodiment of a novel sensor head. For this, a sensor head as shown in Fig 1 to 6, Fig. 21 or Fig. 23 is additionally integrated into a unit which makes it possible to detect simultaneously whether the sensor is reliably in contact with the tissue and whether the contact pressure of the sensor is not too high, and thus represents a pressure indicator.

This unit can be referred to as a secure sensor applicator. It comprises a groove which is 1 to 2 mm wide and in which two pairs of light guides are opposite one another on each side of the groove. On one side two transmitting fibers, on the other side two detecting fibers. The transmitted light from each transmitting fiber is preferably amplitude-modulated with a different frequency in each case. This amplitude modulation makes the secure sensor applicator secure against interference by foreign light sources and, moreover, permits separation of the light intensities coming from the two transmitting fibers.

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The method for calculating the relative hemoglobin concentration  $Hb_{conc}$  in the NIR is to be designed in a similar way to that developed for the visible wavelength range. However, account must be taken of the particular spectral characteristics in the NIR. The selection of the wavelength range considered is crucial for the calculations with the Fitt algorithm because the Fitt algorithm can be applied only if there is an appropriately characteristic spectral difference

depending on the hemoglobin oxygenation. It has emerged that the wavelength range 600 - 900 nm is particularly suitable for measurements in the NIR wavelength range.

Fig. 15 shows the dependence of the backscattered intensity on the one hand on the transmitter-detector separation (x gradient) and on the depth of penetration (z gradient). The x gradient of the backscattered intensities as a function of the transmitter-detector separation and the z gradient can be determined in a scattering cuvette as shown in Fig. 16 without marking ink.

The effective depth of penetration, also referred to as detection depth herein, can be determined by the unit shown in Fig. 16 (see A. Krug, Thesis, Erlangen University, 1998).

Fig. 16 shows a scattering cuvette filled with scattering suspension and marking ink for definition with depiction of the scanning directions. A new light guide separation  $z$  was set with a micrometer screw in each case and was then scanned in the  $x$  direction. The scan represents the light intensities of the various distances from the light guides to the marking ink - the "black hole".

Fig. 17 shows a survey picture of calculations of detection depths using a 90% threshold in various Intralipid suspensions and with various separations, evaluated at 760 nm.

Now to the description of the calculation, which is novel according to the invention, of the current measured volume in the VIS and in the NIR: the measured volume  $V_{\text{meas}}$  can be determined from the measurement of a surface intensity gradient. In addition, a transfer function which must be determined experimentally for the particular tissue is required.

As is to be seen in Fig. 18, the transfer function produces the relationship between the intensity gradient on the surface, which can be established by measurement techniques, and the unmeasurable intensity gradient in the depth of the tissue. The effective measurement volume is regarded as being the volume in which the backscattered intensity gradients have been 90% attenuated, as shown in Fig. 15. The transfer function can be formally described by:

$$I_z \text{ gradient}(z) = \text{transfer function}^{-1} * I_x \text{ gradient}(x)$$

Fig. 19 shows the definition of the determination quantities a, b and c of a semiellipse to illustrate the measured volume formed by a light waveguide on illumination of a scattering tissue.

The measured volume can be determined approximately according to the invention from the determination of the detection depths in the x and z direction (see Fig. 15).

Assuming the measured volumes are semielliptical, the effective measured volume emerges according to Fig. 19 as:

$$V_{\text{Meas. eff.}} = \frac{2}{3} \pi a b c$$

The determination quantities of the ellipse a, b and c can be found by calculating the effective depths of penetration from the intensity gradients. Because of the rotational symmetry of the illumination it is possible to set

$$a = b = x_{\text{eff}},$$

the effective depth of penetration  $x_{\text{eff}}$  in the lateral direction. The depth c can be determined from the effective depth of penetration in the transverse direction, and

$$c = z_{\text{eff}}$$

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is determined via the effective depth of penetration in the  $z$  direction, which is to be directed perpendicularly into the tissue. The 3 parameters of the semielliptical volume integral and thus the relevant measured volume are thus determined.

In the study by A. Krug [Krug, Thesis, Erlangen-Nuremberg University, 1998] it was proposed that the measured volume decreases as the absorber concentration increases. It is possible to show by correcting the measured backscattered extinctions by the reduced measured volume in each case that a linear relationship between the amount of absorber in the scattering suspension and the extinction relative to the current measured volume is obtained even with measurements in highly scattering media.

A method for determining the arterial oxygen saturation by means of a broad-band tissue spectrometer including all the spectral information is proposed according to the invention.

In a first approach, the arterial saturation of hemoglobin is determined by the usual pulse oximeter method with evaluation of at least two wavelengths, there being formation of a pulse-synchronous difference signal of the heart beat. These wavelengths should preferably be selected so that depth-selective determination of these arterial values is also possible, and so that the outputs of the monochromatic and of the broad-band light source are effective in an additive manner at these wavelengths. In a first approach, the conventional pulse oximeter method is included in the integrated sensor head.

A novel approach according to the invention is to determine the arterial saturation from the broad-band backscattered spectra of the tissue photometer. The tissue photometer allows the current hemoglobin saturation in the measured volume to be calculated via the method described above for determining the

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Fig. 20 shows two exemplary spectra. The 80% saturated spectrum corresponds to a state at the end of systole when the content of fresh oxygen-rich blood is greater than at the time of the period of the slower, diastolic perfusion during which the saturation essentially corresponds to the capillary venous saturation.

The blood volume  $\Delta Hb_{\text{amount, art.}}$  is formed from the difference in the amounts of blood and hemoglobin at the end-systolic and end-diastolic time point. The method for hemoglobin determination as described above is used in this case too.

magnetic resonance images, which are very easily accessible to the trained eye of the physician. Information summarized in the form of images additionally provides an excellent possibility for transmitting a large amount of information with a high degree of order. A method for recording initially two-dimensional and subsequently three-dimensional images of the local distribution of the oxygen parameters which can be measured with the sensor described above is presented here.

The sensor technique for recording the various local oxygen parameters as listed in Table 1 has been explained in the preceding sections and is to serve as basis here. Differing from the sensor technique described above with point detection of the oxygen parameters, a novel, primarily two-dimensional imaging method is explained below.

Fig. 21 shows the design in principle of the recording apparatus according to the invention for two-dimensional imaging of oxygen parameters. In this variant, the core of the sensor consists of an imaging light guide (also called imaging bundle) as already used in endoscopes or catheters in order to pass image information from the interior of the body to the outside. The imaging light guide is composed of a bundle of single fibers which are, however, arranged so that the image information is retained. This imaging light guide is arranged between the instrument and the measurement point on the object. In the measuring instrument, this imaging light guide is then scanned with the point measuring probe placed, as described above, directly on the object. The immense advantage of this arrangement derives from the fact that all the moving parts can be attached in the measuring instrument, and the sensor, in this case the end of the imaging light guide, can be fixed directly on the surface to be measured. The novel imaging sensor needs to be fixed only once. The scanning in the instrument means that the object is not displaced by the scanner movement itself, and it is now only necessary to scan

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the flat surface on the instrument side of the imaging light guide. This surface can be scanned with greater speed because the mechanical conditions therefor can be defined better in the instrument.

It is crucial that the individual fiber diameters in the imaging light guide are less than or equal to the cross sections of the point measuring probe. This ensures that the same sensor geometry is always produced by the imaging light guide as previously also defined directly by the point sensor. The imaging of the point sensor increases in accuracy as the density of the packing of the fibers increases and the thickness of the individual fibers of the imaging light guide decreases. The second alternative, exact 1:1 coupling of the individual fibers of the point sensor in each case into exactly one individual fiber of the imaging light guide is also possible but far more complicated.

It is also possible according to the invention to use a plurality of point sensors at the same time. This requires a multichannel illumination source and a multichannel detection unit. This makes it possible to reduce the time for a complete image because, if the point sensors are arranged side by side, for example in the y direction, it would then be necessary to carry out only half the number of scans in the x direction.

As an alternative to the imaging light guide, or as replacement for a scanner and the complete detection unit, it is also possible to use other cameras such as CCD cameras with an additional unit, which make direct spectral analysis of the detected information possible. These additions to the cameras have not to date made adequate spectral resolution possible, but this might soon be realized with further technical developments.

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Fig. 22 shows the image of a scanned perfused liver surface, which demonstrates the local distribution of the hemoglobin saturation. In this method, which is still very elaborate, the point sensor was still advanced directly to the liver surface. Uncoupling of the movement of the point sensor from the organ surface was achieved by a PVC film stretched in between. The image is that of the distribution of the hemoglobin saturation on an isolated perfused liver surface. In accordance with the compilation of optical methods in Table 1, it is also possible to obtain images of the other oxygen parameters and the other tissue pigments mentioned in Table 3.

It emerged from these experiments that the sensor geometry is crucial for the spatial resolution with which the distribution of the oxygen parameters can be imaged. An appropriate sensor resolution appropriate for the morphological structure of the tissue which is to be investigated should therefore be selected via the fiber diameter, aperture and fiber material.

A three-dimensional recording method for imaging local oxygen parameters is built up on the two-dimensional recording method. As shown in Fig. 23, a similar type of imaging light guide (also called imaging bundle) as depicted in Fig. 21 is used. However, in contrast thereto the imaging light guide is now according to the invention scanned in the instrument with the depth-selective sensor from Figure 1 with an x-y scanner. The two-dimensional imaging is combined with the depth-selective recording of the oxygen parameters via the sensor with a plurality of channels differing in separation both for the Doppler measurements and for the detection of the backscattered intensities.

One embodiment of the invention also permits determination of other tissue parameters with the spectrometric measurement methods described above:

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Under physiological tissue conditions, measurements of other pigments at the same time as hemoglobin are very difficult because the absorption caused by the hemoglobin completely obliterates the spectra of the other pigments mentioned. However, determination of the tissue pigments mentioned in addition to hemoglobin is of physiological and clinical interest. Determination of the tissue pigments mentioned is possible in organs perfused free of hemoglobin or in pathologically altered situations. It is possible according to the invention to measure the redox state of the cytochromes, for example during organ transplantation, in the hemoglobin-free state. It is also possible to investigate the myoglobin oxygen saturation and concentration in skeletal and heart muscle.

The importance of monitoring these tissue substances is that this opens up the possibility of being able to investigate directly the intracellular oxygen supply conditions. The Fitt algorithm described above can be converted to calculation of the redox state of the cytochromes investigated by providing completely reduced and completely oxidized cytochrome reference spectra (see Fig. 24). Fig. 24 shows cytochrome

It is possible in the same way, by providing completely oxygenated and completely deoxygenated myoglobin spectra, also to calculate the myoglobin saturation with oxygen via the Fitt algorithm described above.

The wavelength range from 500 to 650 nm is particularly suitable for determining the cytochrome and myoglobin levels with abovementioned methods because, in this wavelength range, the naturally low absorption coefficients of these cellular absorbers have the comparatively highest values. It is thus possible in this wavelength range to attain the clearest absorption spectra with the best signal-to-noise ratio.

The method for determining concentrations of an absorber present in the tissue can, of course, also be extended to artificially injected dyes. It is thus possible for color influx and efflux curves to be measured noninvasively and locally in the tissue directly.



To determine the oxygen content, only the output signals of the broad-band tissue backscattering spectrometer are evaluated. The oxygen saturation ( $SO_2$ ) is determined from the tissue spectra via the color information, and the hemoglobin concentration ( $Hb_{conc.}$ ) is determined from the attenuation of light. The tissue levels can be measured depth-selectively by the multichannel tissue spectrometer signals measuring at various separations, and be related to the relevant measured volume, which is determined on line. Multichannel spectrometer measurement is crucial because this measuring configuration allows the  $SO_2$  values and/or the  $Hb_{conc.}$  values to be related to the measured volume. The measured volume is determined by gradient measurements and subsequent determination of absorption and scattering. Measurements at the same site and time through the same sensor (see Fig. 1) are crucial in order to be able to ensure relation of the signals to the particular measured volumes.

To determine the oxygen consumption in arterial/venous mixed tissue, the output signals of the tissue spectrometer are evaluated together with the pulsatile signals either from a pulse oximeter or from a fast tissue spectrometer. In the case of pulsatile tissue spectrometer measurement, the largest saturation values are evaluated differentially during a cardiac cycle in order to be able to discriminate only the arterial blood contribution. The high data acquisition rate is crucial in order to be able to detect the arterial circulation pulse. Without discrimination of the pulse it is impossible to determine any arterial  $O_2$  saturation. Triggering of the measurement time point for the arterial determination is defined from the laser Doppler signals and/or the time-resolved signals of the  $Hb_{conc.}$  determination. This innovative calculation method for determining the arterial saturation is based on evaluation of the signals from a fast broad-band tissue spectrometer (<20 ms per clock period) and/or the triggering via the signal of the laser Doppler unit.

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To determine the locally transported amount of oxygen, once again multichannel output signals from the tissue spectrometer, the oxygen saturation signal, the hemoglobin concentration, the output signals from the laser Doppler and the blood flow rate or the blood flow must be combined together in order to achieve maximum accuracy. Determination at the same site and time of the tissue spectrometer signals

and the laser Doppler signals through the same probe in measured volumes which are thus comparable is crucial for obtaining these signals. Measurements at the same site and time through the same sensor (see Fig. 1) are crucial in order to be able to ensure relation of the signals to the particular measured volumes.

The oxygen consumption rate in arterial/venous mixed tissue is a relative numerical measure dispensing with an absolute relation of the  $Hb_{conc.}$  and  $SO_2$  to the measured volume. It is therefore possible in this case also to carry out single channel spectrometer and pulse oximeter measurements. The output signals of the tissue spectrometer ( $SO_2$  and  $Hb_{conc.}$ ) and the pulse oximeter signals or the pulsatile differential output signals of the tissue spectrometer (arterial  $SO_2$ ) are combined and evaluated synchronously. Measurements at the same site and time through the same sensor (see Fig. 1) are crucial in order to be able to ensure relation of the signals to the particular measured volumes.

To determine the oxygen turnover, the primary signals of the broad-band tissue spectrometer ( $SO_2$  and  $Hb_{amount}$ ), of the pulsatile tissue spectrometer, of the pulse oximeter ( $SO_{2\ art.}$ ) and/or the laser Doppler primary signals ( $v_{blood}$ ,  $Amount_{erys, moving}$ ) are evaluated in a multichannel manner in order to be able to achieve the relation to the measured volume and/or the depth selectivity of the values. The oxygen turnover indicates the difference in the volumetric flow of  $O_2$  delivered arterially and transported away venously. Measurements at the same site and time through the same sensor (see Fig. 1) are crucial in order to be able to ensure relation of the signals to the particular measured volumes. In addition, determination of the measured volume through spectral intensity gradients is crucial for relating the numerical measure of amount to the joint measured volume.

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Methods for determining the arterial oxygen saturation by differential evaluation of the time-resolved spectral backscattered signals as shown in Fig. 20 with the aid of a fast broad-band tissue spectrometry are also according to the invention. It is also possible to combine with a laser Doppler system for triggering the arterial flow pulse time point for

determining the oxygen saturation with the largest, that is to say arterial, saturation contribution. The advantage of this combination is that the spectrum recording rate of the tissue spectrometer now necessary is thus only relatively low.